High content imaging without sacrificing coverage, resolution or speed: Functional validation through multiple assays

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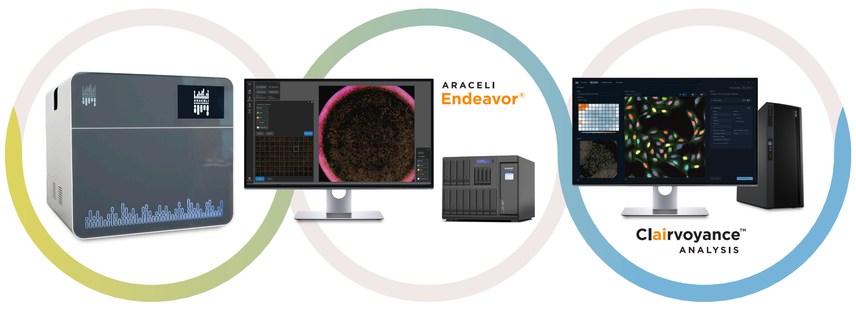
Overview

Validating an end-to-end high throughput solution for high content imaging. Araceli Endeavor® can image 96- 384- and 1536- well plates in <10 minutes, without sacrificing image quality or sample coverage. To match these speeds while taking advantage of this submicron resolution and maximized well coverage, Clairvoyance™ analysis software uses a combination of AI, machine vision, and traditional techniques to quantify these images at speed. Imaging and analysis are seamlessly linked through 25Gbps fiber connections and a 110Tb network attached storage device. This poster highlights validation of this end-to-end system at the level of the bioassay, using a range of common high content assays to quantifiably demonstrate sensitivity, breadth, and broad applicability.



Introduction

Cell-based imaging assays usually involve sacrificing some combination of sample coverage, resolution and speed. Araceli Endeavor® offers high throughput high content imaging without compromise:

- 0.27µm/pixel digital resolution: sub-micron level detection
- Maximized well coverage: eliminate variability and find rare events
- <10 minutes to image entire 96- 384- or 1536 well plate

Araceli Clairvoyance™ analysis uses a combination of machine vision, AI and traditional analysis techniques to yield fast and accurate results, with plate and well visualization to provide confidence in those results.

This poster presents validation of this end-to-end system at the assay level, showing diverse assays from imaging to results in 5-30 minutes:

- Genotoxicity: true submicron-level detection with 2 different outputs
- NFκB translocation: classic subcellular high content assay
- Autophagy 2 ways: compare subcellular puncta quantification of IHC vs live cell dye aggregation, arriving at similar results
- Live/dead: 6 minutes from imaging to a graph of cell positivity

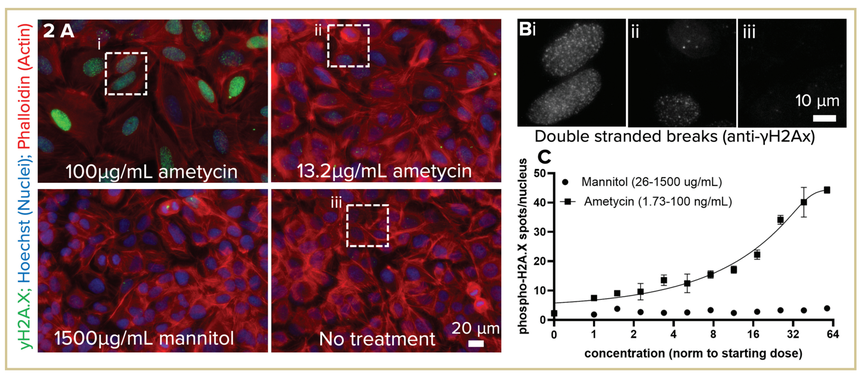
High throughput claims are tested as well, with imaging and translocation analysis done for 45x1536-well plates in a normal workday.

Methods Overview

All experiments use human epithelial cells (A549 or U2OS) in 96- or 1536- well high content plates. Cells were fixed with 10% buffered formalin for 10 minutes prior to imaging. For IHC experiments, fixed cells were permeabilized with 0.1% Triton, and incubated for an hour with 1% BSA before staining. Hoechst 33342 used to visualize nuclei.

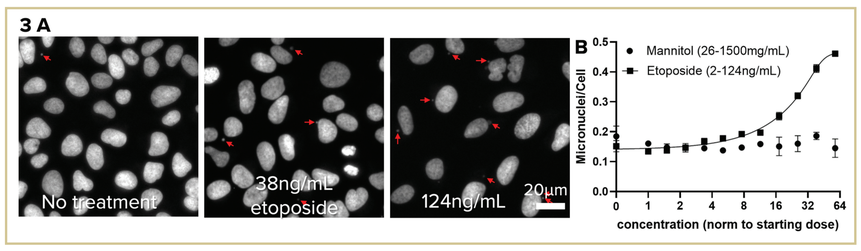
Imaging: Araceli Endeavor® with maximized well coverage, submicron resolution.
Analysis uses Araceli Clairvoyance™: images were vignette corrected, nuclei and spots detected with template match, segmentation with intensity and AI (cell) and watershed/intensity (nuclei and spots).
Graphing in Graphpad Prism, shown +/- standard deviation with 5 parameter logistic curves (solid lines).

Genotoxicity: Sub-Micron Imaging and Nuclear Spot Quantification in <20 Minutes/Plate

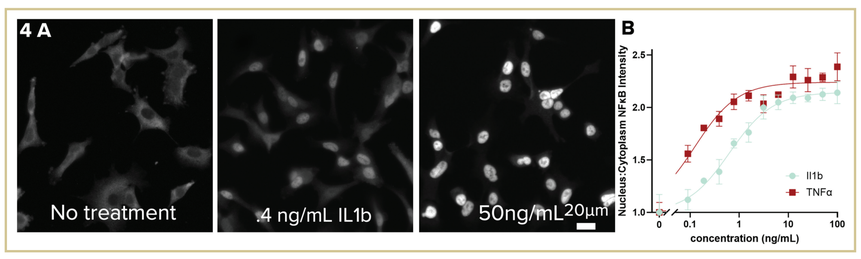


To assay for genotoxicity, cells were incubated with two drugs known to cause double strand breaks in DNA, ametycin (mitomycin c) and etoposide, and inactive control mannitol for 48 hours then incubated overnight with anti-γH2Ax (CST 2577). Imaging took **6 minutes 40 seconds** and nuclear puncta counted per cell in <10 minutes to count nuclear puncta per cell (**Figure 2**). Imaging revealed dose-dependent γH2Ax puncta (**A** with ROIs indicated by dashed boxes in **B**). Dose response plotted (**C**) yielding EC50=16.8 ng/mL (R^2=0.96) and **Z'=0.87**, indicating robust, consistent effect.

More complex analysis was performed in <16 minutes/plate, with nuclear and spot morphology characterized, and actin used to generate a cytosolic mask, which allowed for characterization of extranuclear DNA micronuclei (**Figure 3**). A dose response curve was generated for micronuclear detection with etoposide yielding EC50=23.6µM, R^2=0.99 with **Z'=0.73** for the assay.

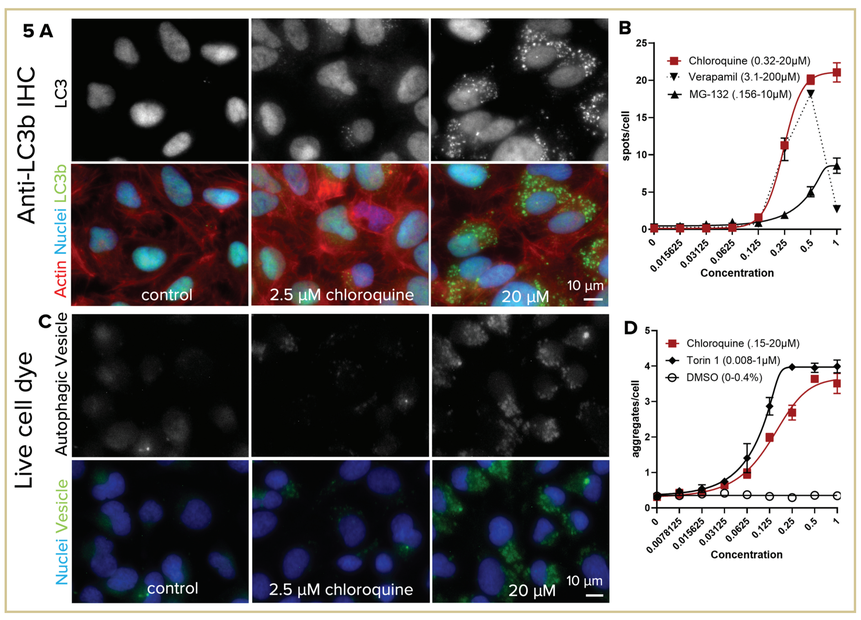


NFκB Translocation After Cytokine Treatment



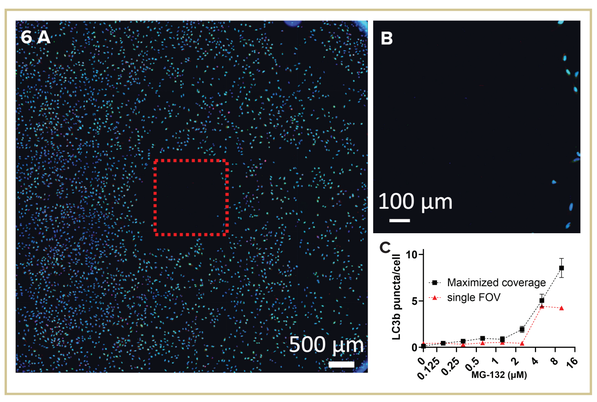
To measure activation of transcription factor, cytosolic to nuclear translocation was measured for NFκB, which upon activation moves from the cell body to the nucleus. Cells were challenged with one of two cytokines, IL-1b or TNFα, for 35 minutes, then stained with a GFP-conjugated antibody against NFkB (CST 8242) for 1 hour. **Figure 4A** shows NFkB response after no, 0.3, and 50 ng/mL IL-1b treatment. Nuclei were detected and segmented based on Hoechst signal, then identified nuclei used as seed for cellular segmentation based on NFkB signal, and ratio of green intensity in the nucleus vs cytosol used to generate **Figure 4B**, yielding EC50 values of 0.65 (IL1b) and 0.13ng/mL (TNFα), R^2>0.94.

Measuring Autophagy 2 Ways: 15-Minute Live Cell Dye Imaging and Analysis vs LC3b IHC



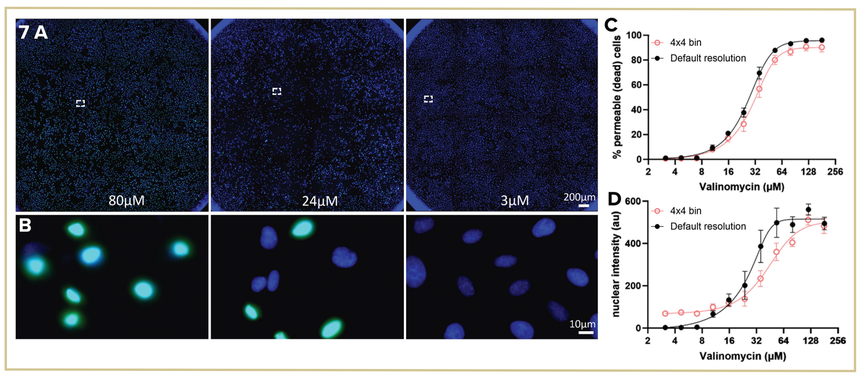
Autophagic flux was modulated through overnight application of autophagy inducers (Torin-1, Verapamil) or vesicle degradation blockers (chloroquine/CQN, MG-132). Autophagy was visualized using 2 different techniques: immunohistochemistry (IHC) against an autophagosomal marker (anti-LC3b, LifeTech L10382 **Figure 5A, B**) or a live cell dye optimized to stain autophagosomal vesicles (Enzo KIT175 **Figure 5C, D**), imaged in **6 min 9 sec** for the whole 96-well plate. For IHC data, LC3b puncta were quantified (**B**) within the cytosol (actin segmentation minus segmented nuclei) in <20 minutes/plate, yielding robust effects with **Z'=0.97** for verapamil and **Z'=0.62** for MG-132. For the live cell dye, nuclei and aggregated vesicles were quantified in **8 min 7 sec**, yielding EC50=0.1µM for Torin-1 with R^2=0.99 and **Z'=0.83** (**D**). While staining and effect size differed between assays (**Figure 5**), both methods showed robust effects with comparable EC50 values (**table**).

Chloroquine	Z'	EC50	R^2
LC3b IHC	0.80908	4.85µM	0.998
CytoID Live	0.71802	2.72µM	0.985



Maximizing well coverage (**6A**) eliminates potentially misleading artifacts seen with a single 1x1mm field of view/well (red box, inset in **B**). Measuring LC3b puncta/cell in the center FOV of the MG-132 dilution series results in non-representative data (**C**), compared to maximized well coverage.

Live/Dead: Cell Positivity Results in 6 Minutes



Cell positivity was measured with the live/dead assay with maximized well coverage in 2 channels (**Figure 7**) using Image-iT DEAD GREEN (Invitrogen) after 24-hour treatment with ionophore valinomycin. After full resolution imaging in **4 min 40 sec**, counting green nuclei (**7C, Z'=0.95**) and measuring nuclear intensity in green (**D**) yielded EC50 values of 26.7 or 26.3µM, respectively, with R^2>0.96 for both, near identical to the kit publisher's EC50 value of 25.5µM. As submicron resolution is not need for this assay, days later this was reimaged with a 4x4 pixel bin in **3 min 30 sec**, with object-level analysis taking **1 min 41 sec** (**C** and **D**, red lines) with an EC50 value of 30.2µM and R^2>0.96.

How many full wells can be imaged then analyzed in a workday?

69,120 wells imaged and analyzed from 9am-5:30pm

- 45 1536-well plates, 276,480 images, 34,340,499 cells
- Image 4 channels, 80% well area, 0.27µm/pixel optical resolution
- Read and write directly to network (NAS)
- Analysis: segment nucleus and cell, measure intensity 4 channels, ratioing nuclear and cellular intensity, yielding object-level data

Discussion

Four distinct, well characterized assays are imaged and analyzed at high throughput speeds, yielding Z'>0.5 with dose response curves.

- Whole plate imaging with 0.27µm/pixel and maximized well coverage in 4-10 minutes
- Object-level analyses interrogating most of the well in 2-20 minutes/plate
- Submicron-level resolution paired withmachine vision-based detection results in robust, consistent effects in genotoxicity
- Well coverage matters: imaging a single field of view leads to different results than imaging a majority of the well
- If resolution is not needed, binning delivers a simple cell positivity assay from loading a plate to analyzed data in 6 minutes
- Assay flexibility: 2 ways of looking at autophagy look different, but yield similar results
- End-to-end submicron imaging with 80% well coverage and subcellular, object-level analysis done for >69,000 wells in a workday

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